Freezing Tolerance of Chicory and Narrow-Leaf Plantain

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ABSTRACT

'Ceres Tonic' and 'Grasslands Lancelot' narrow-leaf plantain (*Plantago lanceolata* L.) and 'Grasslands Puna' chicory (*Cichorium intybus* L.) have received considerable interest as potential new forages for the northeastern USA because of their reported drought tolerance and high forage quality. However, all three cultivars were developed under New Zealand conditions and may not have sufficient winter hardiness to survive northeastern USA winters. Growth chamber and field studies were conducted to determine the freezing tolerance and winter survival of these new forages under well-watered and drought conditions. Winter survival of chicory in the field ranged from 73% of marked plants in the wet treatment to 93% following summer drought. Likewise, winter survival of plantain in the field increased from 3% in the wet treatment to 41% in the dry treatment. Survival of Lancelot plantain in the growth chamber increased from 4% in the well-watered to 16% in the drought treatment. However, survival of Tonic plantain and Puna chicory in the growth chamber was not affected by drought. Chicory survival was greater than survival of plantain in both controlled and field environments. Puna chicory appears to have sufficient winter hardiness to survive winters in the northeastern United States. Although Lancelot had slightly greater survival than Tonic, neither plantain cultivar had sufficient freezing tolerance to be recommended for use in the northeastern USA. Improved cultivars will need to be developed from populations that have evolved under more severe winter conditions before plantain can become a viable forage for most of this region.

IMPROVED CULTIVARS of forage chicory and narrow-leaf plantain have received increasing attention as possible forage and pasture species for the northeastern United States because of their reported high productivity during periods of drought (Stewart, 1996; Belesky et al., 1999; Kunelius and McRae, 1999) and high nutritive value (Jung et al., 1996; Stewart, 1996; Barry, 1998; Belesky et al., 2000). Although naturalized populations of chicory and plantain exist throughout the northeastern USA, the improved cultivars that are currently available were selected for pasture production under New Zealand conditions (Rumball, 1986; Stewart, 1996; Rumball et al., 1997). Sanderson et al. (2001) reported stand losses of 20 to 50% for chicory grown in central Pennsylvania and found that plantain died out completely within 2 yr in one experiment. Plant counts in the spring showed that winter survival of Tonic plantain was low (Labreveux et al., 2001). Stand persistence can be a function of many factors, including stress tolerance, competitive ability, and winter survival. Continued investigations of the freezing tolerance of chicory and plantain are needed to determine the suitability of these species for regions with harsh winter climates.

Some studies have suggested that the presence of neighboring species can affect plant growth and survival in cold environments. For example, nurse-plant canopies appear to protect young saguaro cacti {*Cereus giganteus* Engelm. [= *Carnegiea gigantea* (Engelm.) Britton & Rose]} from freezing temperatures in the winter (Nobel, 1980), while sheltered snow gum (*Eucalyptus pauciflora* Sieber ex Spreng.) seedlings had higher photosynthetic rates and lost less leaf area during winter than exposed seedlings (Egerton et al., 2000). We could find no information on how interactions among forage species might affect freezing tolerance and winter survival.

Drought and freezing tolerance have been closely linked. A primary cause of freezing injury is cellular dehydration caused by water movement to ice crystals that form in intercellular spaces (Steponkus, 1978; Siminovitch and Cloutier, 1983). Acclimation to drought and freezing share several common physiological responses including osmotic adjustment and accumulation of amino acids (Thomas and James, 1993), increased soluble protein and phospholipid content (Cloutier and Siminovitch, 1982b), and dehydrin accumulation (Rinne et al., 1999). Moisture stress (Cloutier and Siminovitch, 1982a) or abscisic acid applications (Mantyla et al., 1995; Gusta et al., 1996) can induce cold hardening in the absence of low temperatures. It is not clear, however, if drought stress can confer an additional level of freezing tolerance to plants that have also undergone the normal cold hardening process.

We investigated the freezing tolerance and winter survival of chicory and plantain growing in monocultures or in multiple-species mixtures to determine if they are sufficiently winter hardy to persist in the northeastern USA. We also investigated the influence of prior drought treatments on the subsequent freezing tolerance of these species. We hypothesized that drought stress would increase the winter hardiness of chicory and plantain compared with well-watered plants.

MATERIALS AND METHODS

Growth Chamber

Seeds of Grasslands Puna chicory and Ceres Tonic and Grasslands Lancelot plantain were germinated at room temperature in petri dishes, transplanted at 10 seedlings per pot

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Abbreviations: PAR, photosynthetically active radiation.

to 15-cm diameter clay pots filled with potting soil, and transferred to controlled environment chambers. Seedlings were sown either as monocultures or as components of five-species/cultivar mixtures containing randomly selected assemblages chosen from alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.), orchardgrass (*Dactylis glomerata* L.), timothy (*Phleum pratense* L.), chicory, Tonic plantain, and Lancelot plantain. Each species or cultivar was included in two different mixtures. For monocultures, a single pot containing 10 seedlings constituted an experimental unit. For the mixtures, an experimental unit consisted of five pots, each pot containing two seedlings of each of the five species in the mixture. Pots were randomly arranged within each chamber. Four growth chambers were used in the study with each chamber constituting a replication. The experiment was repeated once for a total of eight replications. Experimental design was a 2 by 2 factorial with mixture complexity and water treatment as random effects. Preplanned orthogonal contrasts were used to compare species/cultivar means. The comparisons were chicory vs. plantain and Tonic plantain vs. Lancelot plantain.

Chamber conditions were initially set at a 14-h photoperiod with a photosynthetically active radiation (PAR) level of 975 μmol m^{-2} s^{-1} provided by a combination of high pressure sodium, metal halide, and incandescent lights. Chamber temperature regimes were designed to mimic natural diurnal fluctuations, slowly ramping up from a minimum of 15°C at 600 h to a maximum of 20°C at 1400 h then back to 15°C by 600 h the next morning. Air temperature was monitored near the top of the plant canopy. Relative humidity also ramped from a high of 75% at 600 h to a low of 50% at 1400 h, then back to 75%. Pots were watered every 1 to 2 d as needed to prevent water stress. One watering each week was with half-strength Hoagland's solution. Single-leaf transpiration rates were measured with a Li-Cor model Li-1600 steady state porometer (Li-Cor, Inc., Lincoln, NE).

Seedlings were grown under these initial conditions for 3 wk, then water was withheld from half the pots until transpiration in the droughted plants was reduced to <50% of well-watered controls. Pots were rewatered to field capacity and the drought cycle was repeated two more times. Each drought cycle lasted 4 to 5 d. At the end of the third cycle, all pots were watered to saturation and the cold hardening process was started. Chamber temperatures were gradually reduced across a 2-d period to a maximum/minimum of 4/2°C, photoperiod was reduced to 12 h, PAR was reduced 50% by turning off half the lights, and humidity control was turned off. With the controls off, humidity ranged form 75 to 85%. This initial hardening period lasted for 2 wk, then temperatures were reduced for an additional week to a maximum/minimum of 2/−1°C, with a 10-h photoperiod. Chamber temperatures were then reduced to −3°C and ice nucleation in the plants was induced by sprinkling the surface of the pots with crushed ice. After 24 h at −3°C, the temperature was reduced at a rate of 1°C h^{-1} to −14°C. Chambers were kept at −14°C for 3 h, then the temperature was increased to 4°C at the rate of 2°C h^{-1}. After 8 h at 4°C, chamber conditions were returned across a 2-d period to their original, pre-hardening settings for a 3-wk recovery period. At the end of 3 wk, each individual plant was rated for survival and vigor of growth. Vigor scores were 0 (dead), 1 (some green tissue visible but future survival still questionable), 2 (vigorous regrowth with some signs of freezing damage), and 3 (no visible sign of freezing injury).

Field Study

The presence of Puna chicory and Tonic plantain in a preexisting field study at the Russell E. Larson Agricultural Research Center near Rock Springs, PA, allowed winter survival to be monitored as part of a larger experiment that examined the response of several species mixtures to variable moisture conditions on a Hagerstown silt loam soil (fine, mixed, semiactive, mesic Typic Hapludalfs). On 4 Aug. 1999, 1.2- by 1.2-m plots containing several simple and complex species mixtures were established in the field under two automatic rainout shelters. Plots were sown at 1040 seeds m^{-2} with equal numbers of seeds per species. Chicory was included in a five-species mixture containing orchardgrass, perennial ryegrass (*Lolium perenne* L.), Kentucky bluegrass (*Poa pratensis* L.), and white clover. Tonic plantain was included in another mixture containing tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass, Kentucky bluegrass, and red clover (*Trifolium pratense* L.). The rainout shelters were set to move over the plots whenever it rained, excluding all natural precipitation from the plots from 9 May to 4 Oct. 2000. A drip irrigation system provided moisture during the summer at rates based on long-term Central Pennsylvania records equal to a 1- in 10-yr drought (12 mm wk^{-1}), a normal summer (21 mm wk^{-1}), and a 1- in 10-yr excessively wet summer (28 mm wk^{-1}). Soil moisture was measured weekly with a Troxler nuclear gauge (Troxler Electronic Laboratories, Inc., Research Triangle Park, NC).

Plots were mowed to a 6-cm stubble on 4 Apr. 2000 then clipped to a 6-cm stubble height whenever mean canopy height reached 25 cm. Regrowth intervals ranged from 14 to 56 d, depending on season, irrigation treatment, and species composition of the mixture. Even though clipping dates varied among treatments, all plots were clipped five times during the season except the droughted mixture containing plantain, which was harvested four times. Growth rates (kg ha^{-1} d^{-1}) were calculated by dividing harvested biomass by the number of days since the last harvest. Plots harvested during May, July, and September were hand separated by species, and growth rates were calculated for each species individually.

On 31 Oct. 2000, 10 chicory and 10 plantain plants per plot were tagged for winter survival determinations. Maximum and minimum temperatures during the winter were collected from a weather station located at the Russell E. Larson Farm. Snow cover data for State College, PA, were obtained from AccuWeather, Inc. (http://www1.accuweather.com). Marked plants were evaluated for winter survival soon after the beginning of green up in the spring (5 to 9 Apr. 2001). Plants were rated as either dead or alive and percentage survival was calculated. Plots were replicated four times, and arranged in a split plot design with irrigation treatments as the main plots. Species mixtures were randomized within each irrigation treatment. Replications were blocked with two replications under each rainout shelter.

RESULTS

Growth Chamber

When averaged across genotypes, drought treatments in the growth chambers reduced plantain and chicory transpiration rates by 69% in Cycle 1, 77% in Cycle 2, and 66% in Cycle 3 (Table 1). Well-watered Lancelot plantain had significantly lower transpiration than well-watered Puna chicory at the end of Cycle 1 and significantly higher transpiration than both Tonic plantain and Puna chicory in the dry treatment in Cycle 2. When data were combined across drought cycles, Tonic plantain and Puna chicory had the greatest reduction in

Table 1. Chicory and plantain single leaf transpiration rates at the ends of three 4- to 5-d drought cycles in a controlled-environment chamber.

	Cycle 1		Cycle 2		Cycle 3	
	Wet	Dry	Wet	Dry	Wet	Dry
	$\mu g\ cm^{-2}\ s^{-1}$					
'Tonic' plantain	4.2	1.1	4.8	0.4	4.7	1.9
'Lancelot' plantain	3.7	1.4	4.5	1.9	4.2	1.2
'Grasslands Puna' chicory	5.1	1.5	4.5	0.8	4.6	1.5
LSD 0.05	1.4		1.1		1.3	

transpiration in response to drought (75 and 73%, respectively) while Lancelot plantain had the least (61%).

Preplanned contrasts showed that freezing tolerance was greater for chicory than for plantain ($P < 0.01$). Lancelot plantain tended to have greater freezing tolerance than Tonic ($P = 0.08$). Plantain survival in the well-watered pots was <5% and did not differ between cultivars (Fig. 1). Drought stress prior to freezing increased the survival of Lancelot plantain compared with the well-watered control and increased the survival of Lancelot compared with drought-stressed Tonic plantain. Chicory survival was not affected by the drought treatment in the growth chamber study. There was no difference in the survival of plants of either species growing in monocultures compared with those growing in mixtures (data not shown).

All surviving plantain plants received a vigor rating of 1 except for two Lancelot plants in the dry treatment. For most of the plantain rated as *alive*, it was still questionable after the 3-wk recovery period as to whether they would fully recover from the freezing treatment. Average vigor scores for the surviving chicory were 1.5 for plants growing in mixtures vs. 1.3 for plants grown in monoculture ($P = 0.02$). The drought treatment had no effect on chicory vigor.

Field Study

Moisture in the top 30 cm of the soil profile was reduced by ≈30 g kg^{-1} in the drought treatment compared with the normal and wet treatments which did not differ from each other (data not shown). Drought significantly reduced chicory growth rates in September compared with the normal treatment, whereas plantain growth rates in the dry and normal treatments were lower than the wet treatment in July and September (Table 2). Plantain was a relatively minor component of the mixture in which it was planted, comprising only 1% of the harvested biomass in May but increasing to 10% in September. Plantain growth rates tended to increase as the season progressed, especially as soil moisture increased. Chicory, on the other hand, was a dominant component of the mixture in which it was planted, increasing from 31% in May to 62% in September. Chicory growth rates were more uniform during the growing season, although they tended to decrease slightly during July. Irrigation treatment had no effect on species composition in either mixture.

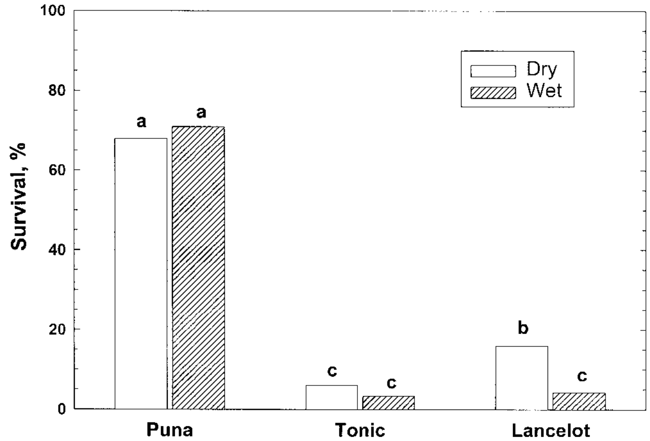

Fig. 1. Survival of Puna chicory and Tonic and Lancelot plantain seedlings exposed to well-watered or drought conditions prior to freezing at −14°C. Data from monocultures and mixtures are combined. Bars with the same letter are not significantly different at $P = 0.05$.

Nighttime air temperatures first approached freezing in early October and by mid-November were below zero on most nights (Fig. 2). Subzero nighttime temperatures continued for most days through the end of March with a minimum temperature of −19°C occurring on 23 Jan. 2001. A particularly cold period occurred between 18 Dec. and 4 Jan. when daytime temperatures did not exceed 0°C and nighttime temperatures reached a minimum of −17°C. Total snowfall during the winter of 2000-2001 was 80 cm, which was below the long-term average of ≈100 cm. Snow cover was generally 5 cm or greater during the coldest periods; however, periods of subfreezing temperatures combined with bare ground occurred during late November and early December, during most of the month of February, and after 13 March. Such snow-free periods are typical of Central Pennsylvania winters.

Winter survival in the field was similar to freezing survival in the growth chamber, with chicory exhibiting much greater survival than plantain (Fig. 3, $P < 0.01$). Winter survival for both species was greatest in the dry treatment ($P = 0.04$), although plantain appeared to be more responsive to drought than was chicory. There was no difference in survival between the normal and wet treatments. Winter kill essentially eliminated plantain from the normal and wet treatments.

Table 2. Effect of variable water applications on daily growth rates of field-grown 'Grasslands Puna' chicory and 'Tonic' plantain growing in five-species mixtures. A moveable rainout shelter excluded all natural precipitation while a drip irrigation system provided 28 mm (wet), 21 mm (normal), or 12 mm (dry) of water per week. Data are ±1 SE.

	May			July			September		
	Dry	Normal	Wet	Dry	Normal	Wet	Dry	Normal	Wet
	$kg\ ha^{-1}\ d^{-1}$								
Chicory	28 ± 8	32 ± 7	34 ± 9	20 ± 5	25 ± 7	25 ± 7	30 ± 7	47 ± 8	37 ± 9
Plantain	0.6 ± 0.3	0.5 ± 0.3	0.9 ± 0.3	1.9 ± 0.6	2.1 ± 0.9	5.2 ± 1.5	1.7 ± 0.8	3.4 ± 1.4	6.7 ± 1.6

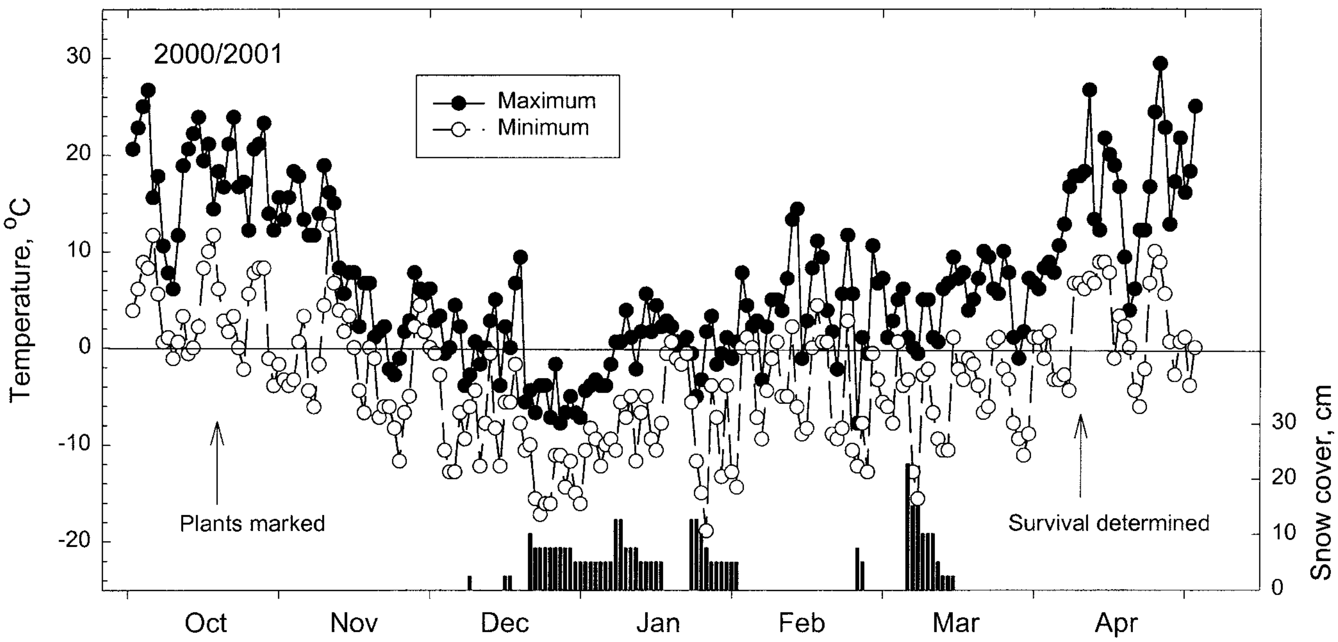

Fig. 2. Maximum and minimum air temperatures and snow cover at Rock Springs, PA, from autumn through spring of 2000–2001 (1 October to 30 April).

DISCUSSION

Although little is known about the freezing tolerance of Puna chicory, Kunelius and McRae (1999) reported that it survived for three production years in the cold-winter region of Atlantic Canada. In that study, temperatures were below normal during the first winter following seeding with mean monthly temperatures in January reaching −12°C. By comparison, the coldest monthly temperatures in our study were −5.8°C in December and −3.4°C in January. December temperatures were 4.3°C colder and January temperatures 1.3°C warmer than normal. Chicory survival was >70% in both the growth-chamber and field studies and exceeded 90% in the field plots that were drought stressed during the preceding summer. Even though Puna chicory was developed under New Zealand conditions and thus was not subjected to winters as severe as those typically encountered in central Pennsylvania, it appears to have sufficient winter hardiness to survive winters in the northeastern USA and Canada.

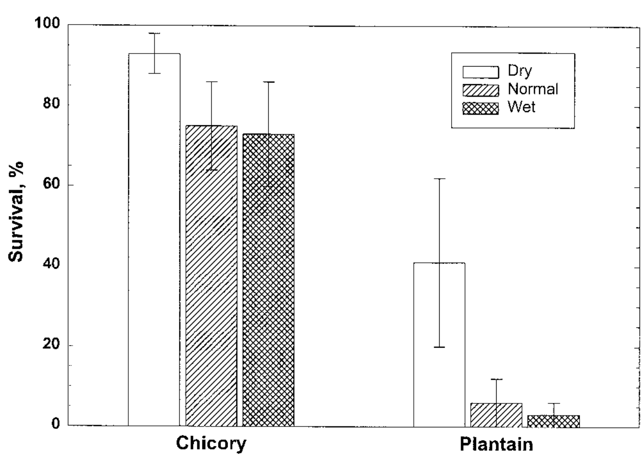

Fig. 3. Effect of moisture stress on the survival of Puna chicory and Tonic plantain following the winter of 2000-2001. Error bars represent ±1 SE.

Li et al. (1997) reported that grazing chicory in late autumn resulted in ≈27% fewer plants the following spring. In our study, the final clipping of chicory occurred on 12 September for the dry and normal treatments and on 5 September for the wet treatment. This was ≈6 wk before the first significant period of subfreezing temperatures began (Fig. 2), and should have allowed sufficient time for accumulation of root C and N reserves for overwintering if chicory behaves similarly to other taprooted species such as alfalfa (Barnes and Sheaffer, 1995). Most producers using management-intensive grazing practices would like to graze into late November or early December if conditions permit. Additional research is needed to determine if continued grazing into the late autumn will adversely affect chicory winter survival.

Although naturalized populations of *P. lanceolata* can be found throughout the northeastern USA, Tonic and Lancelot plantain were developed in New Zealand from germplasm originating in northern Portugal and the north island of New Zealand, respectively (Stewart, 1996). On the basis of our results, neither improved cultivar appears to have sufficient winter hardiness to survive in northeastern pastures. Improved cultivars will need to be developed from populations that have evolved under more severe winter conditions before plantain can be recommended for most of the northern USA.

Plantain grows well under cool temperatures, improving its productivity in dry environments by growing during early spring and late autumn when soil moisture is more readily available (Chatterton et al., 1990). Plantain is valued in New Zealand for its ability to remain green and leafy during winter (Stewart, 1996). Fall dormancy in alfalfa is often closely related to winter survival (Schwab et al., 1996a; Cunningham et al., 1998) such that survival is reduced in cultivars that exhibit vigorous growth during the fall. Plantain growth rates in our study were greater in September than they were in July, increasing by 62% in the normal and 29% in the wet treatment (Table 2). However, in the dry treatment, growth rates were similar in September and July. Imposition of summer drought on plantain increased winter survival from <10% in the normal and wet treatments to 41% in the dry treatment. It is possible that the improved winter survival of drought-stressed plantain was directly related to its reduced fall growth. This hypothesis will be tested in a future study. The improved winter survival of plantain following drought suggests that Tonic and Lancelot plantain might be better adapted to cold-winter regions that are considerably drier than central Pennsylvania.

Studies have shown that drought stress can induce cold hardening in the absence of low temperatures (Cloutier and Siminovitch, 1982a; Mantyla et al., 1995), that transfer of a single stress-inducible transcription factor can improve both drought and freezing tolerance (Kasuga et al., 1999), and that certain stress signaling pathways can be activated by cold and drought (Jonak et al., 1996). Little is known, however, about how the combination of water stress and low temperature interact to affect the cold hardening process. In our study, both Tonic plantain and Puna chicory showed increased winter survival in the field when plants were previously exposed to a summer of continual drought stress. However, in the growth chamber, only the freezing tolerance of Lancelot plantain was significantly improved by drought stress. Survival of Tonic plantain in the growth chamber was only 3 and 6% in the well-watered and drought stressed treatments, respectively, suggesting that the level of freezing stress in the growth chamber was too severe to permit detection of any drought effect that might have existed.

Freezing tolerance of chicory in the growth chamber was not affected by drought (Fig. 1). It is possible that the duration or severity of the drought stress treatment in the growth chamber was not sufficient to induce increased freezing tolerance in chicory. Certainly, plants in the field that experienced several months of drought would have more time to acclimate than would plants that only went through ≈2 wk of drought stress in the growth chamber. Siminovitch and Cloutier (1982), however, demonstrated that winter rye (*Secale cereale* L.) seedlings developed similar levels of freezing tolerance whether they were exposed to 24 h or 2 wk of desiccation stress. Chicory plants in the growth chamber were also younger and smaller than plants growing in the field, which could have potentially affected its response to drought and freezing stress (Steponkus, 1978; White, 1984; Schwab et al., 1996b).

CONCLUSIONS

While Puna chicory appeared to have sufficient freezing tolerance to survive northeastern U.S. winters, Tonic and Lancelot plantain did not, and cannot be recommended for this region. Future use of plantain will require development of germplasm selected from naturalized populations with demonstrated winter hardiness among other traits. Poor freezing tolerance in plantain could be related to its ability to continue growing under low temperatures. This hypothesis will be tested in future experiments. Drought stress improved the freezing tolerance of both chicory and plantain in the field and of Lancelot plantain in the growth chamber, suggesting that these species might be best adapted for winter survival in areas that normally experience periods of summer drought.

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